Molecular Studies to Evaluate Variegations of *Philodendron var. Birkin*

Are Genetic Mutations Responsible for Variegation in *Philodendron Birkin*?

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ABSTRACT

Plant tissue culture and molecular genetic techniques were used to analyze the instability of the genome in philodendron var. 'Birkin.' This plant has variegated, white, green, and greenish-red leaves (that have lost their variegation) on the same plant. Did genetic mutations occur in meristematic sectors of variegated leaves that have reverted to green coloration, or is it an epigenetic change? PCR and sets of RAPD plastid primers were used to determine if the presence or absence of a PCR product correlated with differences in leaf color and variegation (white, green-white, variegated, reddish green or green leaves). A DNA isolation procedure was first optimized to extract PCR-quality plastid DNA from *Philodendron* leaves. A modified DNA procedure (BABEC) was used to isolate plastid DNA. It resulted in a high yield of DNA as measured by the Nanodrop system. PCR was performed with eight different RAPD plastid primers (GB07, GB8, OPA19, OPA22, OPB22, OPC08, OPC12, RAPD primer #1, RAPD primer #2). Differences in PCR products were observed for five primers, while two primers were observed to have no difference in PCR products. This indicated that genetic mutations resulted in differences in leaf color and variegation. We are not excluding the possibility that epigenetic changes also play a role in variegation, however, this has not yet been analyzed. These results could provide useful information in terms of breeding for ornamental traits and introducing improvements to the genus *Philodendron*.

BACKGROUND

In animals, the mitochondrial COI gene reproducibly differentiates most major animal phyla. In plants, however, there is no single DNA barcode that is as reproducible. Sequences typically used to barcode plants are chloroplast sequences, either within coding sequences (such as rbcL and matK) or in intergenic regions, such as trnH-psbA (Normoyle et al. 2022). More than one barcode per individual plant are typically used for taxonomical assignments. Based on this information, it was hypothesized that the eight primers (GB07, GB8, OPA19, OPA22, OPB22, OPC08, OPC12, RAPD primer #2) could also be used to evaluate the presence of chloroplast mutations that result in variegation in Philodendron 'Birkin.'

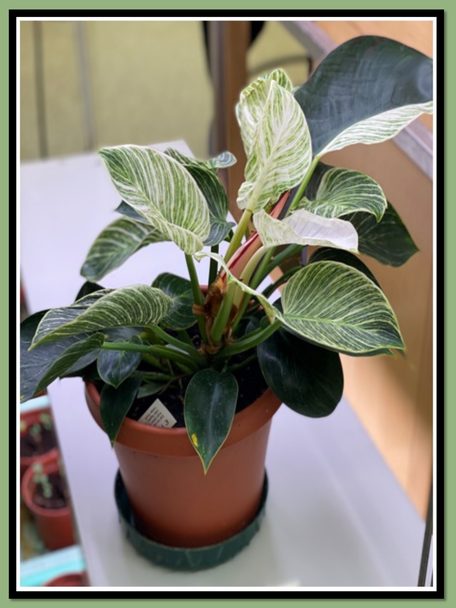

Figure 1. *Philodendron birkin* houseplant used for this study.

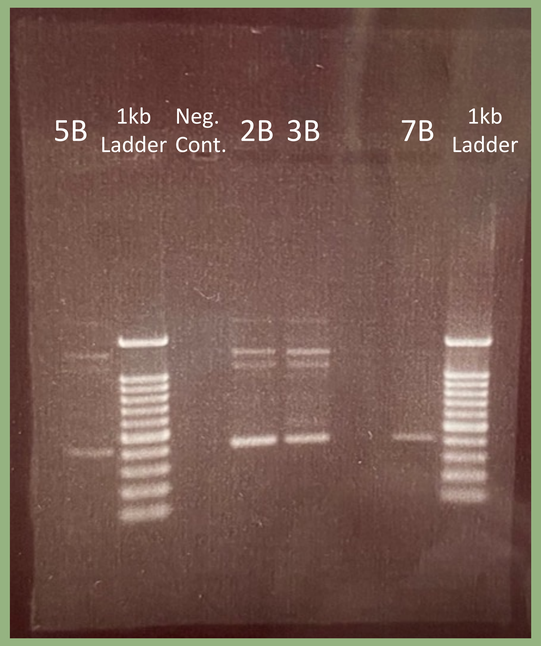

Figure 2. Photo of Electrophoresis Gel of Primer OPA22.

Table 1. Sample ID and correlated leaf type.

Sample	Leaf Type
2B	White
3B	Green Variegated
5B	Reverted Green
7B	Red

Table 2. Primers used to amplify *Philodendron birkin* DNA and the DNA sequences that they target.

Name	Sequence
GB07	5' - GGTGACGCAG - 3'
GB8	5' - GTCCACACGG - 3'
OPA19	5' - CAACGTCGG - 3'
OPA22	5' - TGATCCCTGG - 3'
OPB22	5' - TGATCCCTGG - 3'
OPC08	5' - TGGACCGGTG - 3'
OPC12	5' - TGTCATCCCC - 3'
RAPD primer #2	5' - GTTTCGCTCC - 3'

Table 3. RAPD primer results.

RAPD Primers	Results
# of primers used	8
# of primers producing bands	7
Total # of bands for 8 primers	28
# of Primers producing bands in variegated leaves	7
# of Primers producing bands in green (or older reddish) leaves	7
# of Primers producing bands in white leaves	7

Table 4. RAPD primer DNA fragment sizes.

RAPDS Primer	DNA Fragment (bp)
GB07	1636
GB8	4410, 1224
OPA19	---
OPA22	3558
OPB22	1636
OPC08	2100, 1500
OPC12	2036
RAPD Primer #2	5200

MATERIALS & METHODS

DNA Extraction

- Zymo Quick-DNA Plant/Seed Miniprep kit
- BABEC (https//babec.org) DNA isolation procedure for insect tissue modified for leaf tissue

Nanodrop ND-1000 to estimate DNA concentration and PCR Amplification of DNA Sequences using eight RAPD Primers.

- GB07, GB8, OPA19, OPA22, OPB22, OPC08, OPC12, and RAPD Primer #2
- Each primer targeted a different DNA sequence (Table 2).

Agarose gel electrophoresis

- 1.7 - 2.0% agarose gel
- 10 µL of PCR products
- 10x Orange G tracking dye
- 7.5-10 µL of a 1kb ladder
- DNA fragments were separated at 100 volts, then stained with ethidium bromide strips for 10-15 minutes and photographed with a digital camera and UV filter.

RESULTS

PCR-quality DNA was recovered from white, variegated green, reverted green, and red leaves (that have lost their variegation) using a modified DNA isolation procedure (BABEC). All isolates yielded approximately the same concentration of DNA that had to be diluted 10-fold (to 20 ng/µL) for the PCR. PCR was perhformed with eight different RAPD primers (GB07, GB8, OPA19, OPA22, OPB22, OPC08, OPC12, and RAPD Primer #2), using 3 different thermocycling conditions. Each of te eight primers targeted a different DNA sequence, highlighted in Table 2. Differences in PCR products were observed for the eight RAPD primers (Tables 3 and 4). PCR products were observed for seven of the eight RAPD primers, with OPA19 the only primer that didn't produce PCR results. There were a total of 28 bands that shown with the eight primers. There were seven primers that produced bands in variegated leaves, green (or older reddish) leaves and white leaves (Table 3). Differences in PCR products were observed with five of the eight primers, while two of the primers were observed to have no difference in PCR products.

DISCUSSION

After using molecular genetic techniques to analyze PCR products to compare Philodendron birkin leaves with varying levels of variegation, it was determined that genetic mutation resulted in differences in leaf color and variegation. This conclusion was reached by analyzing the PCR products that showed differences in five of the eight different RAPD plastid primers used. We are not excluding the possibility that epigenetic changes also play a role in variegation, however, this has not yet been analyzed.

In continuing research, we will analyze the role epigenetic changes play in the development of *P. Birkin* variegation. Restriction digests with the methylation-sensitive enzymes HpaI and MspII, followed by PCR analysis will be performed. Restriction digestion will cleave the DNA at locations in which methylation is not present (Zuo et al. 2009). After restriction digestion is performed, if different lengths of DNA are present, it would indicate differences in the methylation of the genes between leaves with different colors of variegation, and epigenetics could then, not be ruled out as a factor playing a role on leaf color.

REFERENCES

Miguel Celia and Liliana Marum (2011) An epigenetic view of plant cells cultured in vitro: somaclonal variation and beyond. J. Exp. Botany 62(11):3713-25.

Normoyle, Caitlin, Shelby Orr, Muira Arndt, and Kimberly Evenson (2022) Molecular Studies to Evaluate Variegation of Philodendron var. Birkin. WSU Rameley Undergraduate Research Symposium.

Temel, Aslıhan, G. Kartal, and Nermin Gozukirmizi. "Genetic and epigenetic variations in barley calli cultures." Biotechnology & Biotechnological Equipment 22.4 (2008): 911-914.

Zuo, Tao, et al. "Methods in DNA methylation profiling." Epigenomics 1.2 (2009): 331-345.